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EXAMINER	
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ART UNIT	PAPER NUMBER
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Below is a communication from the EXAMINER in charge of this application

COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☒ THE PERIOD FOR RESPONSE:

a) ☒ ~~is extended to run _____~~ or continues to run 3 mos. from the date of the final rejection

b) ☐ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for the response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition , and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date of the originally set shortened statutory period for response or as set forth in b) above.

☐ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 7-16-97 has been considered with the following effect, but it is not deemed to place the application in condition for allowance:

1. ☐ The proposed amendments to the claim and /or specification will not be entered and the final rejection stands because:

   a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.

   b. ☐ They raise new issues that would require further consideration and/or search. (See Note).

   c. ☐ They raise the issue of new matter. (See Note).

   d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.

   e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

   NOTE: _____

2. ☐ Newly proposed or amended claims _____ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ Upon the filing an appeal, the proposed amendment ☒ will be entered ☐ will not be entered and the status of the claims will be as follows:

   Claims allowed: NONE
   Claims objected to: NONE
   Claims rejected: 1-4, 8-10, 19

   However;

   ☒ Applicant's response has overcome the following rejection(s): rejections of claims 13-15 are moot due to the cancellation of these claims.

4. ☐ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection because _____

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficent reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☒ Other See attached

PTOL-303 (REV. 5-89)

The period for response CONTINUES to run THREE MONTHS from the date of the final rejection. Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a) accompanied by the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee.

The shortened statutory period for response expires THREE MONTHS from the date of the final rejection or as of the mailing date of this advisory action, whichever is later. In no event however, will the statutory period for response expire later than SIX MONTHS from the date of the final rejection. Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a) accompanied by the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee.

Any extension fee required pursuant to 37 CFR 1.17 will be calculated from the date that the shortened statutory period for response expires as set forth above.

The amendment filed July 16, 1997 under 37 CFR 1.116 in response to the final rejection will be entered upon the filing of an appeal, but is not deemed to place the application in condition for allowance. Upon the filing of an appeal and entry of the amendment, the status of the claims would be as follows:

Allowed claim(s): NONE

Rejected claim(s): 1-4, 8-10, 19

Claim(s) objected to: NONE

REMARKS

The amendment filed July 16, 1997 (paper no. 9) cancelled claims 13-15 and added new claim 19.

The inserted reference to parent application USSN 08/421,079 is **incomplete** because it fails to include the status, i.e. abandoned, of the parent.

The drawings remain objected to for reasons of record (see PTO-948 attached to paper no. 3). The proposed drawing correction and/or the proposed substitute sheets of drawings, filed on July 16, 1997 (paper no. 10) have been approved by the examiner.

NON-ART BASED REJECTION

Entry of the proposed July 16, 1997 amendment upon the filing of an appeal has been approved because the proposal materially reduces or simplifies the issues for appeal. However, proposed new claim 19 raises new issues of clarity (e.g. the difference between "level" and "activity" of adenylate kinase) and antecedent basis (e.g. relationship between the visualization reagent of steps (a) and (b)). Therefore, the examiner would consider a supplemental amendment under 37 CFR 1.116 for the limited purpose of correcting these new problems. The following language, or its equivalent, is suggested for applicant's review.

19. (Amended) A method for determining [the level of] erythrocyte adenylate kinase enzymatic activity in a serum sample comprising the steps of:

(a) determining [the] total adenylate enzymatic activity in a first aliquot of the serum sample by mixing the serum sample with [an] a first adenylate kinase-specific visualization agent which reacts with the total adenylate kinase causing a change in absorbance in the mixture, the change in the absorbance being indicative of the total adenylate enzymatic activity;

(b) calculating [the percentage] percent of the erythrocyte adenylate kinase [to] in the total adenylate kinase in the serum sample by:

(1) electrophoresing a second aliquot of the serum sample in a gel matrix so that the erythrocyte adenylate kinase migrates to a known location on the gel matrix;

(2) contacting the gel matrix with [an] a second adenylate kinase-specific visualization reagent which reacts with the total adenylate kinase and causes emission of fluorescence upon exposure of the gel matrix to ultraviolet light;

(3) exposing the gel matrix to the ultraviolet light;

(4) [determining the level of the total adenylate kinase by] measuring [the] total fluoresecent light emitted from the gel matrix

(5) [determining the level of erythrocyte adenylate kinase by] measuring [the] fluorescent light emitted from the gel matrix at the known location of the erythrocyte adenylate kinase migration on the gel matrix; and

(6) calculating the [percentage] percent of the erythrocyte adenylate kinase [to the total adenylate kinase] by dividing [the level of erythrocyte adenylate kinase by the level of the total adenylate kinase] the measured fluorescent light of step (b)(5) by the measured total fluorescent light of step (b)(4); and

(c) multiplying the [percentage] percent of the erythrocyte adentylate kinase by the total adentylate kinase enzymatic activity to give the [level of] erythrocyte adenylate kinase enzymatic activity in the serum sample.

ART BASED REJECTIONS

Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al. (*Journal of Applied Biochemistry*, 5:437-445 (1983)).

Claims 2, 3 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al. (*Journal of Applied Biochemistry*, 5:437-445 (1983)) as applied to claim 1 above, and further in view of Tsuji et al. (Chemical Abstract 86:39099) or Friedrich et al. (*Biochemical Genetics*, 22 (5/6):389-394 (1984)) and, if necessary, further in view of Buth et al. (Biological Abstract 71059076 (1981)).

Claims 4 and 8-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al. (*Journal of Applied Biochemistry*, 5:437-445 (1983)) as applied to claim 1 above, and further in view of Matsuura et al. (*Journal of Biological Chemistry*, 264 (17):10148-10155 (1989)).

The claimed invention is directed to (1) detection of hemolysis and/or conditions producing hemolysis by measuring serum adenylate kinase ; and, (2) determination of serum erythrocyte adenylate kinase activity.

Olsson et al. found that (i) adenylate kinase was concommitantly release with hemoglobin during cell aging; (ii) cell aging results in progressive lysis of erythrocytes; (iii) adenylate kinase was suitable for monitoring cell lysis due to its extreme storage stability; (iv) there was a high degree of correlation between the amount of accumulated hemoglobin and adenylate kinase; and, (v) while hemolysis was conventionally measured by measurement of extracellular hemoglobin,

adenylate kinase activity measurement was also a sensitive and convenient way to follow hemolysis. An advantage of measuring adenylate kinase lies in studying the lysis of other cell types, e.g. platelets (see page 437, Table I, page 445). Olsson et al. determined adenylate kinase activity in plasma by measuring formation of ATP from ADP by the firefly luciferase reaction. DAPP, which is a specific inhibitor of erythrocyte adenylate kinase, confirmed the origin of the adenylate kinase in the plasma to be erythrocytic (page 442). Thus, Olsson et al. differ in detecting hemolysis by determining erythrocyte adenylate kinase activity in plasma rather than in serum. However, it would have been obvious to one of ordinary skill in the art to modify the method of Olsson et al. by determining erythocyte adenylate kinase activity in serum rather than plasma because serum and plasma are conventional alternative samples used in clinical analysis derived from whole blood.

Olsson et al. also differ in failing to disclose alternative methods for determining erythrocyte adenylate kinase activity, e.g. including the use of gel electrophoresis and immunochemistry, which differentiate adenylate kinase activity of erythrocytic origin from adenylate kinase activity from other cells. Tsuji et al. measure erythrocyte adenylate kinase by agarose thin-layer gel electrophoresis with tetrazolium (i.e. formazan) visualization. Friedrich et al. describe electrophoretic separation and visualization of human erythrocyte adenylate kinase. Buth et al. use NAD-dependent glucose-6-phosphate dehydrogenase in adenylate kinase enzyme staining/detection procedures because it is significantly less expensive than utilizing NADP. Matsurra et al. describe immunoblot analysis of human erythrocyte adenylate kinase (AK1).

Thus, it would have been further obvious and well within ordinary skill in the art to measure erythrocyte adenylate kinase by any known and conventional assay therefore, including electrophoretic separation and staining, such as with NAD-dependent glucose-6-phosphate dehydrogenase visualization technique, immunoassays, etc. as suggested by Tsuji et al., Friedrich et al., Buth et al. and/or Matsurra et al.

Arguments and Rebuttals

Applicant argues (1) Olsson et al. does not detect and distinguish serum erythrocyte adenylate kinase from adenylate kinase of other origins; (2) non-erythrocytes, i.e. platelets, release erythrocyte adenylate kinase, which non-erythrocyte erythocyte adenylate kinase is also inhibited by DAPP; and (3) Tsuji et al., Friedrich et al., Buth et al. and Matsuura et al. determine total adenylate kinase NOT erythrocyte origin adenylate kinase.

In response, (1) it is respectfully submitted that Olsson et al. do distinguish that portion of total adenylate kinase which is due to erythrocyte adenylate kinase from adenylate kinase of other origins by addition of DAPP, which is a specific inhibitor of erythocyte adenylate kinase (see page 442, ¶3). Indeed, the major thrust of Olsson et al. is drawn to erythrocyte adenylate kinase as shown by the constant attention drawn to erythrocyte adenylate kinase and the correlation between cell lysis, hemoglobin release and adenylate kinase release in red blood cells. (2) Olsson et al. state explicitly on page 442, ¶3 that DAPP is **a specific inhibitor of erythrocyte adenylate kinase**, not platelet adenylate kinase. (3) Insofar as Tjusi et al. teaches electrophoretic separation and visual defintion/detection of the adenylate kinase **isoenzymes**, i.e. adenylate kinase

from different cellular sources, it is respectfully submitted that Tjusi et al. suggest determination of erythrocyte adenylate kinase levels (see new claim 19 which also recites electrophoretic separation and determination of erythocyte adenylate kinase). Fig. 2 in Friedrich et al. shows "Histochemical staining of human erythrocyte adenylate kinase". Buth et al. does not specifically address adenylate kinase isoenzymes. However, it is respectfully submitted that one of ordinary skill in the art would have found that suggestion implicit in Buth et al. by virtue of (a) the reference to "electrophoretic" staining procedures and (2) the reference to enzymes commonly resolved by electrophoresis into their respective isoenzymes, e.g. creating kinase. As to Matsuura et al., this reference teaches separation of adenylate kinase isoenzymes, e.g. AK1 (erythrocyte adenylate kinase), by column chromatography prior to determination of the enzymatic activity of each fraction eluted from the column. Therefore, these arguments are not convincing of patentability for the above reasons and reasons currently of record.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carol A. Spiegel whose telephone number is (703) 08-3986.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Paula K. Hutzell, can be reached on (703) 308-4310. The fax phone number for this Group is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Carol A. Spiegel
August 2, 1997

Carol A. Spiegel
CAROL A. SPIEGEL
PRIMARY EXAMINER
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